ANNEX: Technical guidance update on quality assurance for HIV rapid diagnostic tests

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NOTE: the following guidance does not apply to HIV exposed infants less than 18 months of age.

Summary and key points

Quality assurance for HIV rapid diagnostic tests – Critical in the era of ART initiation based solely on HIV serological diagnosis

HIV testing and diagnosis at the point-of-care is an essential part of prevention efforts and a critical entry point into the HIV care and treatment cascade. WHO has supported the use of HIV rapid diagnostic tests (RDTs) within national validated HIV testing algorithms, and has encouraged their utilization by non-laboratory health care personnel and lay providers as critical for the scale up of HIV testing, prevention of mother-to-child transmission (PMTCT), and HIV care and treatment services (1-3). While considerable effort and resources have focused on expanding and decentralizing HIV testing services (HTS), complementary quality assurance approaches for HIV RDTs have not been adequately prioritized or implemented thereby increasing the risk of misdiagnosis. Specifically, both false positive and false negative results have been reported across programmes and countries (4-8). This has raised concerns that some individuals might be incorrectly diagnosed as HIV-positive and started on ART unnecessarily.

The continued expansion and decentralization of HIV testing, care, and treatment advocated by the WHO 2013 Consolidated ARV Guidelines and the UNAIDS’ “90-90-90” by 2020 targets (9) necessitates complementary quality assurance measures be implemented to ensure that individuals receive a correct HIV status. This is particularly critical in settings initiating ART based solely on HIV serologic diagnosis such as under Option B+ and for all children less than five. Specifically, all HTS should require:

- testers trained to follow the national validated algorithm,
- implementation of a standardized logbook or test register documenting key quality elements (i.e. kit names, lot #, expiration dates, result of each test in the algorithm, final interpretation, and results of regular quality control (QC)
- use of a second reader to reread RDT results when possible
- retesting prior to enrollment in care and/or ART initiation to verify an HIV-positive diagnosis, (retesting those on ART is not recommended)
- regularized quality control of test kits
- external on-site quality assessment and supervision at all testing sites
- supply chain reliability

The purpose of this update is to identify approaches, resources and tools that can assist in the implementation of these robust quality assurance measures by HTS, PMTCT, and care and treatment

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programmes to ensure that HIV RDTs are conducted correctly and that an accurate HIV status is consistently ascertained.

9.1. Justification

The WHO 2013 Consolidated ARV Guidelines recommend the immediate provision of ART for all HIV-positive children under 5 years of age, pregnant and breastfeeding women, discordant couples, patients with active TB, and patients with hepatitis B virus (HBV) and severe liver disease. Accurate HIV testing has always been important; however, it has taken on new significance as initiation of ART for these populations is based solely on the status assigned by HIV RDTs without additional immunologic or virologic testing (i.e. CD4 or viral load) where eligibility thresholds have historically applied. The consequences of misdiagnosis will only increase as more population groups are identified for ART initiation based solely on HIV serological diagnosis. The problem is compounded in low prevalence settings given that the positive predictive value of HIV testing decreases as prevalence decreases.

<table>
<thead>
<tr>
<th>Misdiagnosis:</th>
<th>Occurs when persons without a disease (i.e. those HIV-uninfected) are classified as diseased (i.e. HIV-positive) and vice versa. Misdiagnosis may be a function of the testing strategy or the testing algorithm, poor test kit performance, or operator error.</th>
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<tr>
<td>Repeat testing</td>
<td>refers to a situation where additional testing is performed for an individual immediately following a first test during the same testing visit due to invalid or discrepant test results; the same assays are used and, where possible, the same specimen.</td>
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<td>Retesting:</td>
<td>There are three different types of retesting that WHO recommends within HIV programmes: (a) retesting people at on-going risk for HIV infection (for example, in settings of high HIV prevalence and incidence, pregnant women in their third trimester or in the breastfeeding/post-natal period and at least annual retesting for key populations); (b) retesting people with inconclusive test results after 14 days; and (c) retesting to verify HIV-positive diagnosis prior to enrollment in care and/or ART initiation.</td>
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When quality assurance measures are implemented the performance (sensitivity and specificity) of WHO prequalified HIV RDTs performed by both laboratory technicians and trained HIV testing providers in resource limited settings is excellent (10-12). However, the use of highly sensitive and specific assays is not enough to ensure that an accurate status is always given. Comparisons of sentinel surveillance of antenatal care (ANC) populations using enzyme immunoassay (EIA) testing with HIV diagnosis in PMTCT programmes using HIV RDTs have revealed discrepancies in HIV status (both false positive and false negative). Mozambique (13) found low (88.5%) positive percent agreement of HIV status from their PMTCT programme versus their surveillance test results. Similarly, Kenya found poor agreement between their PMTCT programme data compared to their surveillance data. These discrepancies might be attributed to use of different testing strategies, and different selection of assays used within testing algorithms (14). Although the extent of misdiagnosis is uncertain, the accumulation of evidence suggests that HIV misdiagnosis occurs.

While the majority of test results generated by HIV RDTs are correct, the consequences associated with a misdiagnosis reinforce the need for strengthened quality assurance. The false HIV-positive diagnosis and the initiation of life-long ART for truly HIV negative individuals poses an unnecessary burden on the individual and the programme, and may result in unnecessary side effects of ART, individual stress due to stigma, and loss of confidence in HIV testing in the community. There are also serious individual and public health

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4 Children less than 18 months of age continue to have nucleic acid testing (NAT) as their HIV diagnostic assay
implications for people who receive a false HIV-negative diagnosis including missed opportunities to diagnose 
HIV, inability to maximize health outcomes by early enrollment into treatment and inability to prevent 
ongoing transmission to infants and partners.

Causes of misdiagnosis
The causes of misdiagnosis are multifactorial and include errors at multiple points along the diagnostic 
continuum (See Box 9.1A). At national level, testing strategies that do not take into account national 
prevalence can lead to incorrect results. Because the positive predictive value of diagnosis declines with HIV 
prevalence, it is critical that the correct testing strategy be followed on the basis of prevalence to avoid 
misdiagnosis. At site level, as highlighted in a South African external quality assessment, deviation from the 
national testing algorithm (often due to unavailability of one of the assays), not consistently and accurately 
following all aspects of the testing procedure, and failing to perform QA (quality assurance) activities might 
lead to a decrease in the assay sensitivity and specificity (15). Robust quality assurance programmes are 
designed to detect and correct these errors and therefore minimize the risk.

Box 9.1A Causes of misdiagnosis

<table>
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<tr>
<th>Common sources of errors that might lead to misdiagnosis</th>
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<tr>
<td>• Poor selection of assays to construct the testing algorithm(s) (e.g. low specificity of second line and/or third line assay);</td>
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<tr>
<td>• Deviation from the national validated testing algorithm (e.g. using the second line or third line assay as the first line assay);</td>
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<tr>
<td>• Deviation from the instructions for use issued by the manufacturer (e.g. not waiting long enough or waiting too long for the required incubation time, or incorrect amount of buffer or specimen applied to the test device);</td>
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<td>• Incorrect conditions for transport and storage of test kits (e.g. higher than recommended temperature or humidity);</td>
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<td>• Incorrect interpretation of test results;</td>
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<td>• Transcription or clerical errors;</td>
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<td>• Random errors (e.g. incorrect specimen added to test device).</td>
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Programmatic and operational interventions to improve quality assurance for RDTs
In its 2015 policy guidance, *Consolidated guidelines for HIV testing services (HTS)* (16) and in the *Handbook for Improving the Quality of HIV-related Point-of-Care Testing (POCT): Ensuring Reliability and Accuracy of Test Results* (in press), WHO describes the activities needed in planning, implementing and sustaining quality assurance activities. Additionally, the Interagency Task Team (IATT) on the Prevention and Treatment of HIV Infection in Pregnant Women, Mothers and Children (http://www.emtct-iatt.org/) has created the *Option B+ Toolkit* (http://www.emtct-iatt.org/wp-content/uploads/2015/05/IATT-Toolkit-Dec-2014-JR-1-28-15-Web1) which has a section providing guidance on key approaches to ensure the accuracy of HIV testing.

Programme managers need to work closely with laboratory staff to ensure that these quality assurance activities are implemented in all HTS locations (i.e. ANC, maternity, inpatient wards, TB clinics, outpatient departments, etc.) and that large sites implementing Option B+ are prioritized. With the decentralization of RDTs to thousands of testing sites and community settings, quality assurance programming needs to take into account the remote location of many testing sites (and therefore probably poorer infrastructure), the different types of providers performing the testing, and the large number of sites that need to be included. Resource
limitations may restrict the ability of all elements of quality assurance to be implemented simultaneously. Consequently, a phased approach to the scale-up of quality assurance at each testing site should be considered based on client volume, number of RDTs performed (through-put), presence of large populations initiating ART based solely on the RDT results (e.g. Option B+), and results of any previous QA activities. These activities and recommendations for phased implementation are summarized in Box 9.2A. More detail on algorithm development and retesting is provided below.

**BOX 9.2A: Key activities to strengthen quality assurance for RDTs: Ensuring reliability and accuracy of test results**

<table>
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<th>Phase 1: Required of all national programs and sites</th>
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<tr>
<td>1. National policies and algorithm: Develop national policies and guidelines that assist the implementation of QA activities. Ensure a national validated testing algorithm with a back-up testing algorithm (sufficient to have an alternate for the first assay) for use in cases of stock-outs of RDTs.</td>
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<td>2. Trained testing personnel: Ensure that the national standardized training and certification programme includes a hands-on component and incorporates key quality assurance elements including a comprehensive overview of the testing algorithm, use of QC specimens, correct completion of HIV RT logbook/register, review of the logbook/register for QA purposes, performing proficiency testing, criteria for repeat testing, and basics of RDT forecasting. A system for periodic re-certification should also be implemented.</td>
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<td>3. Adoption of a standardised logbook/register: The national HIV program should work with the NRL to adopt a HIV RDT logbook/register that includes key quality assurance elements (i.e. kit names, lot #, expiration dates, result of each test in the algorithm, final interpretation, and results of regular QC). All testing sites should be required to utilize this register in conjunction with other registers such as ANC, TB, and ART.</td>
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<tr>
<td>4. Supply chain strengthening: Ensure that HIV RDTs and required consumables are reliably available at testing sites and that sites know how to properly forecast and quantify RDT needs and place orders in a timely manner.</td>
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| 5. Quality/process control: To ensure the RDT is operating correctly implement the use of:  
  - Standard operating procedures and job aides  
  - Kit based internal quality controls  
  - External quality control specimens (positive and negative known specimens commercially available or prepared by the NRL and distributed to the sites). |
| 6. Second reader: Where resources allow, a second reader should make a blinded rereading of each RDT, especially in cases where HIV-positive results will be issued. This is standard practice for visually read assays, both for HIV and for other conditions. The second reader needs to be trained only on how to read the assay, not necessarily on the test procedure itself. If the two readers interpret the test results the same way, the test report is issued as is. If the two readers do not agree, HIV testing should be repeated using a new specimen and a new test device. Inter-reader disagreement for RDTs ranges from 0 to 1.6%. |
| 7. Retesting: Implement retesting of newly diagnosed HIV-positive individuals prior to enrolment in care and/or initiation of ART as a part of QA. |
| 8. External quality assessment: On site direct supervision (through direct observation and mentoring) for new testers by experienced testers. |

**Phase 2: For more mature programs where resources allow**
9. **Proficiency testing/external quality assurance programme**: Distribute PT/EQA panels from a provider (e.g., NRL) for blinded testing at testing sites and provide timely feedback and corrective measures to sites participating in the programme.

10. **Documentation for quality assurance**: Implementing methods and indicators to monitor RDT quality at all levels of the health system and in each HTC setting including review of the logbook/register and proficiency testing results. Use this data to identify quality gaps, implement quality improvement efforts, and inform program planning.

11. **Site supervision and certification**: Routine site supervision which includes direct observation of all RDT site operations, review of RDT site documentation and the use of a standardized, scored checklist for site certification.

**Algorithm development and use**

WHO recommends two testing strategies for HIV diagnosis, one for high prevalence and one for low-prevalence settings. Because the positive predictive value of diagnosis declines with HIV prevalence, it is critical that testing strategies be selected on the basis of prevalence to avoid misdiagnosis. An HIV positive status should not be given without two sequential reactive test results in high prevalence (≥5%) settings or 3 sequential reactive test results in low prevalence (<5%) settings.

Strict adherence to the validated national testing algorithm is essential in all HIV testing locations. Deviation from the national HIV testing algorithm due to the unavailability of any of the assays in the algorithm is a common reason for misdiagnosis. A final positive diagnosis (status) can never be given based on a single HIV RDT result. In situations where only the first assay in the algorithm is available, a HIV-negative status can still be given if the test result of the first assay is non-reactive, but if the test result is reactive and if the second and third assays are not available, the client should be referred to another clinic where all assays are available or they should be scheduled to return as soon as the assays are available so that client can be tested following the national validated testing algorithm.

In situations where only the second or third assay of the testing algorithm is available, clients should be scheduled to return or referred to another site where all assays are available. Second and third line assays are selected to be the most specific assays which may be at the expense of sensitivity, thereby increasing the risk of a false negative result.

**Retesting**

The 2013 WHO Consolidated ARV Guidelines recommend that, “Retesting all people living with HIV before initiating ART is good practice to ensure correct diagnosis of HIV infection (page 89).” The 2015 Consolidated guidelines for HTS expand on this recommendation and specify three instances where retesting is recommended to assure accurate diagnosis:

1. retesting people at ongoing risk for HIV infection (for example, key populations at high ongoing risk); given the improved detection thresholds of RDTs, most people testing HIV-negative do NOT need to retest to rule out being in the window period.
2. retesting people with HIV-inconclusive test results after 14 days; and
3. retesting to verify an HIV-positive diagnosis before initiating care, and particularly before starting antiretroviral therapy (ART).

Retesting people who are already on ART is not recommended.

All newly diagnosed persons should be retested by a second operator (preferably at the site where ART will be initiated) using a second specimen and following the same testing algorithm, irrespective of whether or not ART initiation depends on CD4. With the exception of exposed infants 18 months and younger, ART should
not be initiated without the initial diagnosis being verified. Retesting according to this procedure aims to rule out possible technical or clerical errors, including specimen mix-up through mislabeling and transcription errors, as well as random error either by the provider or of the test device. Retesting will not exclude misdiagnosis related to poor choice of a testing algorithm; however, with adequate validation of the testing algorithm, this risk should be minimized.

While some clinics may retest clients who present with a HIV-positive status issued from another facility or from community testing programmes, systematic retesting as a national policy has only been adopted in a few settings. In 2014, Malawi, pioneers of the PMTCT Option B+ approach, added the recommendation to retest all newly identified HIV positive individuals to verify test results prior to ART initiation to their national guidelines (3). The Malawi Ministry of Health started to monitor and report test results in 2014. In the first quarter of monitoring confirmatory status, the MoH concluded that: “This relatively high proportion of clients who did not have a concordant positive confirmation [on re-testing] may be explained by selective confirmatory testing among clients with doubts about their previous positive status. This underscores the importance of both routine retesting before ART initiation as well as the need to continue strengthening HTC quality assurance process (17).”

Médecins Sans Frontières, (MSF) has also implemented systematic retesting to detect any quality issues for HIV testing. MSF noted suboptimal sensitivity and specificity of HIV assays in the field in multiple countries and implemented quality assurance measures and routine retesting prior to ART initiation (18). In order to prevent the problem, training, supervision and quality control were strengthened. The decision was made to introduce retesting when it became apparent that false positive status were still occurring. Training was given to counsellors on helping individuals deal with false positive results using common scenarios and role playing. Specific information about the difference between HIV cure and a false positive result was given in the pre-test counselling to avoid misinterpretation. Psychological support was offered as long as needed, and in some cases, free health care was continued for a limited time for those found to be HIV negative.

Implementing retesting of individuals newly diagnosed as HIV positive requires consideration of the forecasting and resource implications for procurement of RDTs and associated testing consumables. Additionally, it requires strong community messaging emphasizing that retesting those that are HIV positive prior to ART initiation does not imply that testing services are unreliable but rather that it is good clinical and testing practice to double-check the HIV status prior to starting lifelong ART. All communication should explicitly reassure stakeholders and the public that the results of assays, when performed per protocol, are extremely accurate. HIV assays are prequalified by the WHO, and national authorities should validate national algorithms prior to use in programs. Further, HIV testing and counselling protocols should be updated to include clear counselling messages for this situation and procedures for supplementary testing in order to resolve an individual’s HIV status if the test status is not the same upon retesting. HIV testing providers should be retrained on the issues around retesting for verification purposes and the appropriate counselling messages and supplementary testing procedures.

Individuals undergoing HIV testing must be made aware of the risk of incorrect diagnosis, particularly if they do not disclose that they are on ART. All individuals receiving HIV testing should be asked if they have been tested previously and told they are HIV-infected, if they are now on ART, or if they have ever received ART. If the HIV status is the same upon retesting, the individual’s HIV-positive status should be considered as verified. If the test status is not the same upon retesting, the individual or their specimen should be referred for additional testing at a higher-level facility.
Monitoring and Quality Improvement of QA activities

In order to monitor implementation of this retesting recommendation, the 2015 Consolidated Strategic Information Guidelines (19) includes a new quality indicator to assess whether retesting to verify HIV diagnosis prior to ART initiation is taking place. Current logbooks and registers should be adapted to allow for recording results of the retesting event. This will be important not only for routine program monitoring, but also for assessing baseline rates of discordant testing events. The Monitoring & Evaluation Framework for Antiretroviral Treatment for Pregnant and Breastfeeding Women Living with HIV and Their Infants offers enhanced monitoring indicators (use of testing logbook/register, participation in EQAS/PT programme and stock-out of RDTs) that can be used to measure quality of HIV testing. Data should be used continuously for decision making to take corrective action and on when a site is ready to implement the next quality assurance activity.

Ensuring correct diagnoses: estimated cost of retesting pregnant women

In a setting where resources are scarce it is crucial to determine savings programmes may incur from discovering a false HIV-positive diagnosis. Programmes that have implemented retesting of positives have noted that they were able to do so with minimal costs. To illustrate this further take the following example using published costs of HIV testing and ART treatment, and variable rates of misdiagnosis. In resource constrained settings, total discounted lifetime treatment costs of HIV are estimated at US$17,570 per person given a life expectancy of 30 years from ART initiation and median annual ART costs of $880.

Therefore, assuming a rate of misdiagnosis of 4 per 100 persons tested (mean across all RDTs performed), the per person cost of retesting would have to be greater than a threshold of (17570*4/100) = US$703 in order to outweigh the treatment costs associated with misdiagnosis. Economic evaluations suggest that the cost of testing in health care settings using the national validated testing algorithm ranges from US$5-$20 per person, this includes all relevant direct costs such as testing materials, staff time, and administrative overhead. These testing costs are 35 to 140-fold smaller than the aforementioned threshold. Assuming the lowest rate of misdiagnosis, 1.61 per 100 persons tested, the threshold would be US$283 which is 14 times greater than a retesting cost of US$20, or, for every US$20 spent on retesting, US$263 are saved in averted treatment costs.

At US$20 per person tested, the upper end of the range, retesting is favorable in settings where the misdiagnosis rate is as low as 0.11 when retesting with a national validated testing algorithm. These examples indicate that, retesting is far less expensive than lifetime ART treatment of those with false HIV-positive status even in settings where false diagnosis rates are low and retesting costs are high. These calculations assume that retesting would eliminate misdiagnosis and does not consider the mental and psychological costs that may be associated with HIV diagnosis.

With respect to pregnant women, the total cost of retesting all newly diagnosed HIV positive pregnant women will vary based on each country’s HIV positivity rate among pregnant women. In adopting a policy of retesting for all newly diagnosed HIV positive pregnant women, under the most conservative assumptions, a country with 25,000 newly diagnosed HIV positive pregnant women annually would spend (25,000*$20) = US$500,000 on retesting each newly diagnosed pregnant woman and conservatively save in (25,000*1.61/100*$17,570) = US$7,071,925 in averted treatment costs, for net medical savings of US$6,571,925.

Areas of Future Research
1. Quality Corps Volunteer (Q-Corps)
While countries have implemented quality assurance measures at various levels, the coverage still remains limited. To address this gap, several countries are currently piloting the use of community based volunteers who are trained on specific tasks in the quality assurance measures for testing conducted at point-of-care. This new cadre works with the MOH and the National Reference Laboratory to facilitate the roll-out of quality assurance activities at numerous decentralized HIV testing sites. These Q-Corps volunteers are assisting with:

- the logistics of EQAS (proficiency testing) panel distribution, collecting EQAS results, and returning EQAS results back to participating sites with suggested corrective action;
- the logistics of collecting and tallying standardized testing logbook/register for monthly summaries;
- the data analysis of the testing logbook/register data with suggested corrective actions;
- site assessments to identify need for corrective action and onsite training; and
- timely follow-up of implementing quality assurance activities in numerous decentralized sites.

Based on their needs, countries’ approaches vary with respect to the involvement of the community Q-Corps volunteers. Operational research efforts should explore the effectiveness and contribution of Q-Corp volunteers to the health workforce and impact on the quality of HIV testing.

2. Use of electronic image capture for test results, i.e. RDT readers

Use of smart phones and other new technologies for image capture and interpretation of results allows the interpretation of a test result to be verified by an “electronic second reader”. These have been mostly developed utilizing open access software that calculate intensity of the test and control bands, and applying an algorithm to interpret the test result, such as when control band is present and test band is present then the result is valid and can be interpreted as HIV reactive. There are also cell phone-based RDT reader platforms that can work with various lateral flow immuno-chromatographic assays (e.g. Determine). These types of innovative tools may assist testing providers to improve the accuracy of reading test results (20). Considering the possibility of such manufacturing related tools and/or incorrect human reading of RDTs in field conditions, future evaluations should explore whether digital and automated RDTs using smart readers might help to achieve higher efficiency and accuracy.

9.2. Conclusion

Quality assurance activities are required in every HIV testing site. Ministries of Health and National HIV Programmes must prioritize quality assurance and quality improvement efforts for HTS and ensure that national policies, guidelines, and training curricula include recommended best practices. Clinical settings implementing the WHO 2013 Consolidated ARV Guidelines recommending ART initiation based solely on the result of the HIV RDTs without need for CD4 count that reaches a certain threshold should be prioritized for quality assurance activities to prevent individual and public health consequences of a misdiagnosis.

A quality management system should be implemented to conduct quality assurance activities including training and retraining with competency-based assessments, external quality assessment (through direct observation with suggested corrective actions), quality control measures (QC specimens, temperature controls, etc.), rereading of visually read assays by a second operator, and proper documentation and recordkeeping including use of standard operating procedures (SOPs) and analysis of a testing logbook/register at every testing site.

The implementation of an EQAS (proficiency testing) programme should be prioritized to high volume testing sites, prioritizing testing sites that initiate ART without using CD4 for triage and sites with known quality
assurance challenges. External quality assessment via proficiency testing can be combined with site supervision at MCH/PMTCT clinical sites providing HIV testing and to assure accuracy and to provide technical support to operators. Specific quality assurance approaches, such as retesting any newly diagnosed HIV-positive individual prior to enrolment in care and/or starting ART and repeat testing of specimens with originally discrepant test results should be implemented as best practices to ensure all individuals receive a correct HIV diagnosis. Implementation of these recommended quality assurance measures will minimize the potential harm of an incorrect diagnosis and maintain the public’s confidence in HIV testing services. Finally, National HIV Programmes must monitor the quality of testing on a regular basis; otherwise providers will not know whether quality services are being provided in line with the national guidelines.

References

17. Q1 Integrated HIV Program Report, Ministry of Health of Malawi; 2014.